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SUMMARY OF RESULTS FOR OU6 WATER AND SEDIMENT TOXICITY TEST RESULTS

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OU6 Toxicity Section

INTRODUCTION

An important objective of the OU6 characterization is to use an integrated strategy in defining water quality. The EPA authorizes an integrated approach that involves the measurement of water and sediment chemical make-up, whole effluent toxicity (WET), and biological conditions. When the WET and biological monitoring approaches are used, it is possible to gain a better understanding of the additive effects that the water chemistry has on downstream aquatic systems and users.

The OU6 characterization included water and sediment toxicity tests on all OU6 ponds to measure possible contaminant effects on aquatic and benthic organisms. This section will report the toxicity results and discuss the points of interest.

METHODS

Sampling Locations

RFP has performed water toxicity tests from 1989 to present for NPDES permit outfalls (Sewage Treatment Plant (STP), B-5 influent to A-4, A-4 Discharge, and C-2 Discharge) and other ponds in question. Within OU6, there is historic WET data for the STP effluent, B-5, A-3, and A-4 Ponds. To avoid redundancy, the ponds that have not shown a history of water toxicity results were not re-tested. Those excluded from water toxicity tests for this characterization include A-3 and A-4 Ponds.

The locations tested for water toxicity are shown in Table 1. In addition to the ponds, DOE-RFO, EG&G, USEPA, and CDH selected sampling locations in Walnut Creek upstream from the ponds and at positions immediately downstream from significant tributaries (Figure 1). These additional locations were to be sampled during base flow and storm flow conditions.

There is no historic sediment toxicity testing in OU6. All of the OU6 ponds were chosen as areas of interest for sediment toxicity testing due to their downstream location from RFP and sediment loading (Table 1 and Figure 1).

Water and sediment toxicity samples were taken as split samples with chemical analyses for all locations excluding control samples.

Laboratory Methods

There were two levels of water toxicity testing applied to the OU6 characterization: the WET screen and WET dilution series.

The WET screen is an inexpensive test used first to determine whether toxicity exists. The test is simplified with four replicates and a control. In each replicate, five organisms were tested in a non-diluted water sample. The control is made up of reconstituted water. The SeaCrest Group performed the 48-hour tests using *Ceriodaphnia dubia* (water flea) and the 96-hour test using *Pimephales promelas* (fathead minnow). If there was no toxicity for the WET screen, the toxicity testing was completed for that sample site. If toxicity existed, a second sample was taken and tested in a WET dilution series.

For the WET dilutions, water samples were subjected to acute replacement static toxicity tests conducted in conformity with "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" USEPA 600/4-90 027 and the Region VIII USEPA "NPDES Acute Test Conditions - Static Renewal Whole Effluent Toxicity." The WET dilution series is made up of four replicates for a 100% sample, and four replicates each for samples diluted to 75%, 50%, 25%, and 12% of the sample water. Five organisms are tested in

each replicate for each dilution. Again, a control is run with reconstituted water in four replicates. The SeaCrest Group performed the 48-hour test using *Ceriodaphnia dubia* (water flea) and the 96-hour test using *Pimephales promelas* (fathead minnow). The results were reported as the Lethal Concentration 50 (LC₅₀). LC₅₀ is the percent solution resulting in 50% death of the test population versus the control blank.

SeaCrest performed the chronic sediment toxicity tests on *Hyalella azteca* in 28 day exposures and on *Chironomus tentans* in 10 day exposures. ASTM Method E1383-90 described by Nelson et al (1990) was used. The parameters measured, survival and growth, were compared to a sand control to determine significance of results.

The SeaCrest Group was not able to acquire enough *Chironomus tentans* from suppliers to run all of the sediment samples for OU6. The locations successfully tested included SW107, and A-3, A-4, B-3, B-4, and B-5 Ponds.

A large suite of organic, metal, and radionuclide data was gathered on the sediment samples. The analytes examined included 55 organics, 26 metals, and 10 radionuclides.

RESULTS AND DISCUSSION

Water Toxicity

Water toxicity tests for A-3 and A-4 Ponds were not performed because of an historic record of no toxicity. A-5 Pond was not tested because its source water is A-4 Pond. In 1991 and 1993, WET screens for the remaining ponds were run as a part of the OU2 and OU6 characterization and resulted in no toxicity except for B-3, B-4, and B-5 Ponds. B-3, B-4, and B-5 Pond samples exhibited moderate toxicity (Table 2).

These ponds receive STP effluent where ammonia levels are typically high. In these samples, total ammonia ranged from 11-30 mg/L. Unionized ammonia (NH₃) has been demonstrated to be the principle toxic form, not the ammonium ion (NH₄⁺) (EPA 1986).

Unionized ammonia in these samples based on pH and test temperature ranged from 0.3-2.6 mg/L. The EPA Quality Criteria for Water (1986) lists unionized ammonia acute toxicity to 29 fish species from 0.08 to 4.6 mg/L. For 19 invertebrate species acute toxicity ranged from 0.53 to 22.8 mg/L. SeaCrest reports that acute effects occur for *Ceriodaphnia dubia* at 0.86 and - *Pimephales promelas* at 0.3 to 0.5 mg/L (Fucik 1993). Total ammonia and toxicity decrease downstream from B-3 to B-5 due to nitrification/denitrification.

Water toxicity was again tested for the OU6 characterization using the dilution series on B-3 and B-4 in April 1993 due to toxic results in the screen tests. These tests resulted in no measurable toxicity (Table 2). Further B-5 Pond WET dilutions were not performed because of an abundance of historic dilution test results (Table 3).

The base flow toxicity tests were conducted in April and May of 1993. These sites include all locations listed in Table 1 excluding the ponds. For this investigation, the term baseflow is operationally defined to be a hydrologic condition where a single precipitation event is not occurring.

During sampling, four of fifteen locations were dry (Table 2). At all other sample sites the LC₅₀s were greater than 100% which indicates no measurable toxicity.

Storm flow samples were to be taken as splits with the chemistry on May 17, 1993, but due to a miscommunication, the toxicity samples were not taken.

Sediment Toxicity

Table 4 provides the results of the chronic sediment toxicity tests performed by SeaCrest Labs (SeaCrest 1993). Of the samples tested, only two showed a significantly lower survival rate than the sand controls performed in conjunction with the samples. Site SW107 had *H. azteca* survival rate statistically lower than the sand control. SW107 and SW127 were chosen to represent background levels of sediment toxicity found outside the influence of the RFP discharges. SW107 is along the western most boundary of RFP on Woman Creek (Figure 1).

SW107 was sampled because it is out of the direct influence of Rocky Flats yet is within the plant boundary. It is however, not out of the influence of human activities from offsite and may have been impacted from activities along Colorado Highway 93 or up-gradient cattle ranching. Sampling error may also be responsible. Furthermore, this site is different from pond sites in that it is at the head of a drainage which contains water from groundwater seeps. The water is known to be lower in hardness than RFP pond water. The chemical characteristics of this water are likewise different than RFP pond water in that it typically has lower concentrations of metals, organics, and less buffering capacity. However, SW127 which is directly south of SW107 showed no toxicity to *H. azteca*. This sediment should have been very similar to SW107's.

The other site with a significantly lower survival rate for *Hyalella* versus the control was pond B-2. The overall survival was 51 out of 80 organisms. Chemical data on the pond sediments is available to compare with toxicity findings. However, "[t]o assess the importance of types of in-place pollutants one must know more than how much of each chemical exists in the sediment. It is necessary to know the forms in which the chemicals exist and how available they are to benthic organisms or to be transported (sic) in the water column (de Bernardi 1990).

To assess the apparent sediment toxicity in pond B-2, only the total concentrations of sediment associated radionuclides, metals, and organics are known. The speciation or availability of each within the sediment is unknown. So, for a first approach to determine a potential toxin or group of toxins causing B-2 toxicity, the total levels of sediment associated chemicals in B-2 Pond were compared with the levels found in several nontoxic ponds at RFP. This assumes that the fraction of the total value which is actually biologically available is the same in each pond, so their total values can be compared.

B-1 and B-3 Ponds were chosen as the nontoxic comparisons to B-2 since they showed no significant toxicity to *Hyalella*. B-1 and B-3 Ponds are assumed to be very similar to B-2 since they are located approximately 100 yards from B-2, have similar geology, and are within the same watershed. However, B-1 and B-2 are fed only by direct run-off, groundwater infiltration, and precipitation, while B-3 receives effluent from the RFP STP as well as the sources which feed B-1 and B-2. Table 6 illustrates a comparison of the various sediment associated chemicals within each pond.

In examining the concentration of each toxic metal among the ponds, several are higher in B-2 than in B-1 (Table 6). All of the metals except arsenic were at lower concentrations in B-2 sediment than B-3 sediment. However, nontoxic sediment from B-4 Pond had higher concentrations of arsenic than B-2 sediment. Also, summing the concentrations of the toxic metals in each pond sediment, B-2 Pond sediments were lower in total toxic metals than all other B-series ponds. Hence, the sediment toxicity in B-2 is probably not due to metal concentrations (Table 6).

Pond sediments were analyzed for ten anthropogenic and natural radionuclides. Also, gross alpha and beta radiation was measured. Of the radionuclides measured, cesium-137, radium-226, and strontium-89,90 were higher in B-2 sediments than B-1 sediments (Table 6). Gross alpha and beta measurements of the sediment sample from B-2 were lower than the B-1 sample. Hence, radiation is probably not the cause of toxicity in B-2 sediments.

From comparison of the pond sediments, it is apparent that B-2 is the least similar to other ponds in the concentration of organics in its sediments. This is an indication that organic compounds may be the source of toxicity in B-2 Pond. Many of the organics were labeled as unknowns in

that they were not identified by the laboratory performing the analysis and were simply reported as an unknown at a particular concentration. Therefore, from the available data and lack of definitive identification of many of the detected organics, the contaminant or contaminants of concern are not obvious.

Two other observations are noteworthy. An estimated 2 gallons of diesel fuel were spilled into B-2 in 1992 from a diesel powered transfer pump. At least a few of the unknown organics found in B-2 were hydrocarbons. Also, SeaCrest noted that the DO of this sample was among the lowest measured in the suite of samples tested (<1.0) (SeaCrest 1993). However, B-3 Pond had a comparably low DO, but was not significantly toxic.

It is notable that B-5 Pond had a lower overall survival than B-2 in the four replicate tests (48 out of 80 organisms, 20 organisms run per replicate test). However, the B-5 test had a large variance and standard deviation between replicates (Table 5). Statistical comparison (Dunnett's Test) of B-5 results to the sand control showed the differences in survival were not significant.

None of the samples tested showed average *H. azteca* weights significantly lower than the controls for that test.

Survival of Chironomids was not statistically different in the samples versus their sand control. SeaCrest noted the abundance of naturally occurring Chironomids in many of the samples (SeaCrest 1993).

CONCLUSIONS

Water toxicity tests for the pond and drainage sampling sites resulted in acute toxicity to *Ceriodaphnia dubia* and *Pimephales promelas* for three locations: B-3, B-4, and B-5. Unionized ammonia was at toxic levels for these samples. The second testing of B-3 and B-4 Ponds resulted in no toxicity. There is an abundance of historic data for B-5 with high unionized ammonia concentration periodically.

One pond in OU6 resulted in measurable sediment toxicity. B-2 sediments were toxic to *Hyalella azteca*. The *Hyalella sp.* survival rate was significantly lower than the sand controls performed in conjunction with the samples. The distribution of toxicity as well as chemical contamination in B-2 Pond should be examined in detail. Though it appears upon first analysis that organic compounds are the prime interest for understanding toxicity in B-2, other categories of contaminants must not be ruled out. Thorough analysis of the "unknown" organics in B-2 sediments is required. Careful data analysis and literature studies should help illuminate the availability of sediment associated chemicals in B-2 sediments.

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Table C-1 Sampling locations for water and sediment toxicity testing

Location	Water	Sediment (2)	
	<i>Ceriodaphnia/Pimephales</i> (1)	<i>Hyalella</i>	<i>Chironomus</i> (3)
A 1	X	X	NTO
A 2	X	X	NTO
A 3	NA	X	X
A 4	NA	X	X
A 5 (Warren Creek at Indiana)	NA	X	NTO
B	X	X	NTO
B 2	X	X	NTO
B 3	X	X	X
B 4	X	X	X
B 5	X	X	X
SW 16	X	NA	NA
SW 18	X	NA	NA
SW093	X	NA	NA
GS 3	X	NA	NA
SW0918	X	NA	NA
GS12	X	NA	NA
GS11	X	NA	NA
GS03	X	NA	NA
GS09	X	NA	NA
GS10	X	NA	NA
GS103	DRY	NA	NA
SW022	DRY	NA	NA
# 1	DRY	NA	NA
# 2	X	NA	NA
# 3	DRY	NA	NA
Sediment Control SW107	NA	X	X
Sediment Control SW127	NA	X	NTO

NOTES

- (1) NA = Not applicable These locations were not tested due to historic non toxicity
 (2) NA = Not Applicable These locations were not tested for sediment toxicity
 (3) NTO = No test organisms An adequate supply of Chironomids was not available

ie 3. Summary of NPDES biomonitoring data applicable to OU6 from 1989 to present (a)

	STP EFFLUENT			B-5 TRANSFER			A-4 DISCHARGE			A-3 IN-POND		
	Ceno LC50	Fat LC50	Ammonia mg/L	Ceno LC50	Fat LC50	Ammonia mg/L	Ceno LC50	Fat LC50	Ammonia mg/L	Ceno LC50	Fat LC50	Ammonia mg/L
19												
xil												
ne												
ptember	100	100	12 9									
10												
nuary												
nged from quarterly to monthly sampling												
rch							100	100	0	100	100	0
ril							100	100	0	100	100	0
ly							100	100	0	100	100	0
ne	100	73 4	28 3									
ly												
gust												
ptember	100	33 7	50 (b)				100	100	0			
tober	88 2	41 6	26				100	100	0	100	75 9 (e)	0
vember	100	100	22				100	100	0			
cember	60	52 3										
1												
nuary	41 2	18 5	45				100	100	0			
bruary	100	100	27				100	100	5 7			
rch	100	100	21				100	100	11			
xil	94 5	64 2	41				100	100	5 6			
ly	100	95 5	33 8	100(100)	83 9(100)	9(8 5)	100	100	5 7			
ne	100	100	33	100(100)	100(100)	4 5	100	100	2 3			
ly	75 8	47 5	29 3	100(100)	100(100)	7 6(7 6)				100	100	0
gust	100	100	26	100	100	6 6	100	100	3 2	100	100	0
ptember	100(100)	100(100)	14 9(4 7)	100(100)	100(100)	10 3(11 2)	100	100	2 9	100	100	0
tober	100(100)	100(100)	22 8(12 8)				100	100	1 7	100	100	0
vember	100(100)	100(100)	3 6(0)	100	100	13 3				100	100	0
mber	100	86 6	29 3	100	100	12 1	100	100	6 6	100	100	0
nuary	100(100)	78 5(100)	32 4(5 8)	100	100	12 1	100	100	6 6			
bruary	100(100)	100(100)	2(0)	100	91 5	17 8	100	100	9 7			
rch	100(100)	100(100)	26 9(6 4)	100	100	8 4	100	100	7 2			
xil	67 1(100)	79 4(100)	22(1 8)	100	100	6 6	100	100	3 8			
ly	100(100)	41 9(100)	39 2(6 0)	100	100	8 9	100	100	3 3			
continued in pond sampling												
ne	100	{9}	27 9	100	100	10 9	100	100	2			
ly	83 9	100	17 9	100	100	7 1	100	100	0			
gust	100	100	20 8	100	100	7 5						
ptember	100	100	20 4	100	100	4 9	100	100	1 9			
nged from monthly to quarterly sampling												
tober	100	100	17 21	100	100	9 5	100	100	2			
vember												
cember												
13												
nuary	100	83	24 3	100	100	16 8						
bruary							100	100	6 4			
rch												
xil	100	100	18 3	100	100	12 2	100	100	12 1			
ly												
ne												
ly												
gust												
ptember												
tober												
vember												
cember												

The Seacrest Group (formerly T H E Laboratories) processed these biomonitoring tests

Seacrest Group noted this value to be "suspect" expected value is probably half this level"

ue in parentheses e.g (100) is the EC50 after the sample was filtered through zeolite

A value in braces e.g [100] represents the EC50 of a second test within the stated month

The October 1990 fathead EC50 for A-4 B-5 and C-2 were all unusually low and suspect

Zero values for ammonia represent no detection

The lab failed to set up the fathead test

Table 4 Sediment toxicity results summary for OU6 (Compiled from SeaCrest 1993)

RFP Sampling Site	Sampling Date	Contractor Location Code	Sample Number	Toxicity to Hyalella (%Surviving)	Survival Statistically Different Than Control	Toxicity to Hyalella (Ave Weight in mg)	Weight Statistically Different Than Control	Toxicity to Chironomids (%Surviving)	Survival Statistically Different Than Control
Sand Control	11/4/92	NA	NA	89	NA	0.13	NA	82	NA
Sand Control	11/18/92	NA	NA	74	NA	0.06	NA	.	.
RFP Control	11/4/92	SW107	SEDEF	41	Yes	0.09	No	65	No
RFP Control	11/5/92	SW127	SD50012	85	No	0.06	No	.	.
Sand Control	11/4/92	NA	NA	89	NA	0.13	NA	82	NA
Sand Control	11/18/92	NA	NA	74	NA	0.06	NA	.	.
Sand Control	12/4/92	NA	NA	38	NA	0.06	NA	.	.
Sand Control	11/25/92	NA	NA	85	NA	0.05	NA	.	.
Sand Control	10/29/92	SED60392	SD60003	95	No	0.11	No	.	.
A-1	11/12/92	SED60892	SD60008	89	No	0.15	No	.	.
A-2	10/21/92	SED61392	SD60013	76	No	0.1	No	103	No
A-3	10/19/92	SED61892	SD60018	99	No	0.17	No	73	No
A-4	11/19/92	SED64892	SD60048	89	No	0.33	No	.	.
A-5	11/16/92	SED62392	SD60023	91	No	0.16	No	.	.
B-1	11/18/92	SED62892	SD60028	64	Yes	0.14	No	.	.
B-2	10/27/92	SED63392	SD60033	84	No	0.11	No	88	No
B-3	10/22/92	SED63892	SD60038	91	No	0.19	No	62	No
B-4	10/20/92	SED64392	SD60043	60	No	0.12	No	72	No

Availability of Chironomids and sample holding time limits resulted in inability to perform toxicity tests on this organism for the listed samples

NA=Not applicable

Microtox Test was not performed on this sample

Table 5 Comparison of chemical constituents found in B 1 B 2 and B 3 Pond sediments

Location Comparison	Concentration Individual Toxic Metals	Concentration Total Toxic Metals	Concentration Individual Radionuclides	Gross Alpha Radiation	Gross Beta Radiation	Concentration Individual Organics	Concentration Total Organics
B 1 vs B 2	B 2 > in Ag Hg Cs As	B 1 > B 2	B 2 > in Ra 226 Cs-137 Sr 89 90	B 1 > B 2 by 410.3 pCi/g	B 1 > B 2 by 17.12 pCi/g	B 2 > B 1 for 19 (15 were Unknowns)	B 2 > B 1
B-3 vs B 2	B 2 > in As	B-3 > B 2	B 2 > in U 233 234 Ra 226	B 3 > B 2 by 33.1 pCi/g	B 3 < B 2 by 2.91 pCi/g	B 2 > B 3 for 27 (20 were Unknowns)	B 2 > B 3

Table 6 Statistical analysis of Hyalella survival in B 5 Pond SW107 and B 2 Pond

Location	Hyalella Survival Replicate 1 (out of 20)	Hyalella Survival Replicate 2 (out of 20)	Hyalella Survival Replicate 3 (out of 20)	Hyalella Survival Replicate 4 (out of 20)	Total Hyalella Survival (out of 80)	Standard Deviation	Vanance	Dunnett Table Value 1 tailed P=0.05	T Statistic	Significant Difference
Sand Control For B 5 and SW107 tests	16	17	18	20	71	1.708	2.917	2.46 (df=20,6)	NA	NA
B 5	10	18	13	7	48	4.69	2.2	2.46 (df=20,6)	2.233	No
SW107	15	12	5	1	33	6.397	40.917	2.46 (df=20,6)	3.689	Yes
Sand Control for B 2 Test	19	16	14	19	68	2.449	6	2.18 (df=9,2)	NA	NA
B 2	14	13	12	12	51	0.957	0.917	2.18 (df=9,2)	3.72	Yes

US Department of Energy
Rocky Flats Plant
Figure 1
Surface Water
Sample Location

- Gaging and sampling station for storm-event monitoring
- NPDES storm water permit sampling site
- Paved roads
- Unimproved dirt roads
- Streams, ditches, and other drainage features
- Rocky Flats Plant boundary
- Ponds/Lakes
- Buildings or structures

Map scale = 1:20000
1 inch = 1670 feet

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